

*Claims*

- 1 1. A method of preparing a sulfated polysaccharide capable of binding to a binding  
2 partner, comprising treating an unsulfated or incompletely sulfated polysaccharide with at  
3 least one enzyme.
- 1 2. A method of preparing heparan sulfate, comprising treating an unsulfated heparan  
2 synthon or incompletely-sulfated heparan sulfate precursor with at least one enzyme.
- 1 3. A method of preparing a sulfated polysaccharide capable of binding to a binding  
2 partner, comprising treating an unsulfated or incompletely-sulfated polysaccharide with at  
3 least one chemical reagent and at least one enzyme.
- 1 4. A method of preparing heparan sulfate, comprising treating an unsulfated heparan  
2 synthon or incompletely sulfated heparan sulfate precursor with at least one chemical reagent  
3 and at least one enzyme.
- 1 5. The method of claim 3 or 4, wherein the chemical reagent is selected from the group  
2 consisting of an *N*-deacetylation reagent, an *N*-sulfonation reagent, an epimerization reagent,  
3 and an *O*-sulfonation reagent.
- 1 6. The method of any of claims 1-4, wherein the enzyme is selected from the group  
2 consisting of an *N*-deacetylase, an *N*-sulfotransferase, an epimerase, and an *O*-  
3 sulfotransferase.
- 1 7. The method of claim 1 or 3, wherein the unsulfated or incompletely sulfated  
2 polysaccharide is treated with up to four chemical reagents.
- 1 8. The method of claim 1 or 3, wherein the unsulfated or incompletely sulfated  
2 polysaccharide is treated with up to four enzymes.
- 1 9. The method of claim 2 or 4, wherein the heparan synthon or incompletely sulfated  
2 heparan sulfate precursor is treated with up to four chemical reagents.

- 1 10. The method of claim 2 or 4, wherein the heparan synthon or incompletely sulfated  
2 heparan sulfate precursor is treated with up to four enzymes.
- 1 11. The method of claim 1 or 3, comprising:  
2 (a) treating an unsulfated polysaccharide with an *N*-deacetylating reagent;  
3 (b) treating the step (a) product with an *N*-sulfating reagent;  
4 (c) treating the step (b) product with an epimerizing reagent; and  
5 (d) treating the step (c) product with at least one *O*-sulfating reagent.
- 1 12. The method of claim 2 or 4, comprising:  
2 (a) treating a heparan synthon with an *N*-deacetylating reagent;  
3 (b) treating the step (a) product with an *N*-sulfating reagent;  
4 (c) treating the step (b) product with an epimerizing reagent; and  
5 (d) treating the step (c) product with at least one *O*-sulfating reagent
- 1 13. The method of 2 or 4, wherein the heparan synthon is a non-sulfated *N*-acetyl  
2 heparosan.
- 1 14. The method of claim 1 or 3, wherein the unsulfated polysaccharide is isolated from a  
2 cell.
- 1 15. The method of claim 1 or 3, wherein the unsulfated polysaccharide is isolated from *E.*  
2 *coli* bacteria.
- 1 16. The method of claim 2 or 4, wherein the heparan synthon is isolated from a cell.
- 1 17. The method of claim 2 or 4, wherein the heparan synthon is isolated from *E. coli*  
2 bacteria.
- 1 18. The method of claim 11 or 12, wherein the deacetylating reagent is selected from the  
2 group consisting of a deacetylase and *N*-deacetylase- *N*-sulfotransferase.
- 1 19. The method of claim 11 or 12, wherein the deacetylating reagent is selected from the  
2 group consisting of hydrazine and a metal hydroxide.

- 1 20. The method of claim 11 or 12, wherein the *N*-sulfating reagent is selected from the  
2 group consisting of an *N*- sulfotransferase and *N*-deacetylase- *N* sulfotransferase.
- 1 21. The method of claim 11 or 12, wherein the *N*-sulfating reagent is selected from the  
2 group consisting of trialkylamine sulfur trioxide and chlorosulfonic acid.
- 1 22. The method of claim 11 or 12, wherein the epimerizing reagent is selected from the  
2 group consisting of C5-epimerase.
- 1 23. The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 3-*O*  
2 sulfate group.
- 1 24. The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 6-*O*  
2 sulfate group.
- 1 25. The method of claim 11 or 12, wherein the *O*-sulfating reagent incorporates a 2-*O*  
2 sulfate group.
- 1 26. The method of claim 11 or 12, wherein the at least one *O*-sulfating reagent(s)  
2 incorporates one at least one of a 2-*O* sulfate group, a 6-*O* sulfate group, and a 3-*O* sulfate  
3 group.
- 1 27. The method of claim 11 or 12, wherein the *O*-sulfating reagent is a 3-*O*  
2 sulfotransferase.
- 1 28. The method of claim 11 or 12, wherein the 3-*O* sulfotransferase is selected from the  
2 group consisting of 3-OST1, 3-OST2, 3-OST3, 3-OST3a, 3-OST4, 3-OST5 and 3-OST6.
- 1 29. The method of claim 11 or 12, wherein the *O*-sulfating reagent is a 6-*O*  
2 sulfotransferase.
- 1 30. The method of claim 11 or 12, wherein the 6-*O* sulfotransferase is selected from the  
2 group consisting of 6-OST1, 6-OST2, and 6-OST3.
- 1 31. The method of claim 11 or 12, wherein *O*-sulfating reagent is a 2-*O* sulfotransferase.

- 1 32. A method of detecting or identifying a polysaccharide binding partner, comprising:  
2 (a) combining the binding partner with a polysaccharide under suitable conditions for  
3 detecting a binding partner - polysaccharide interaction,  
4 wherein the polysaccharide is prepared according to the method of claim 1; and  
5 (b) detecting or measuring said interaction.
- 1 33. A method of determining the enzymes that participate in the synthesis of a  
2 polysaccharide, comprising treating a polysaccharide one or more of at least one *N*-  
3 deacetylating reagent, *N*-sulfating reagent, epimerizing reagent, and *O*-sulfating reagent and  
4 examining the enzymatic products generated by said treatment; wherein the generation of an  
5 enzymatic product is indicative that the enzyme participates in the synthesis of the  
6 polysaccharide.
- 1 34. The method of claim 33, wherein the order in which enzymes participate in the  
2 synthesis of the polysaccharide is determined.
- 1 35. An enzymatically synthesized *N*-sulfated, 2-*O* sulfated, 6-*O* sulfated, 3-*O* sulfated  
2 heparan sulfate that binds to antithrombin III.
- 1 36. An enzymatically synthesized *N*-sulfated, 2-*O* sulfated, 6-*O* sulfated, 3-*O* sulfated  
2 heparan sulfate that binds to glycoprotein D of herpes simplex virus 1, wherein the 3-*O*  
3 sulfation was made by 3-OST3a.
- 1 36. An enzymatically synthesized *N*-sulfated, 6-*O* sulfated, 3-*O* sulfated heparan sulfate  
2 that binds to antithrombin III.
- 1 37. An enzymatically synthesized Polysaccharide selected from the group consisting of  
2 Polysaccharide 1, Polysaccharide 2, Polysaccharide 3, Polysaccharide 4, Polysaccharide 5,  
3 Polysaccharide 6, Polysaccharide 7, Polysaccharide 8, Polysaccharide 9, Polysaccharide 10,  
4 Hexasaccharide 11, Hexasaccharide 12, Hexasaccharide 13, Pentasaccharide 14,  
5 Pentasaccharide 15, Pentasaccharide 16, and Tetrasaccharide 17.
- 1 38. The method of claim 37, wherein the polysaccharide is a functional analogue or  
2 derivative of a pentasaccharide.

1 39. A method of synthesizing a selected from the group consisting of Polysaccharide 1,  
 2 Polysaccharide 2, Polysaccharide 3, Polysaccharide 4, Polysaccharide 5, Polysaccharide 6,  
 3 Polysaccharide 7, Polysaccharide 8, Polysaccharide 9, Polysaccharide 10, Hexasaccharide 11,  
 4 Hexasaccharide 12, Hexasaccharide 13, Pentasaccharide 14, Pentasaccharide 15,  
 5 Pentasaccharide 16, and Tetrasaccharide 17.

1 40. A method of purifying a polysaccharide comprising the steps of isolating the  
 2 polysaccharide using mass spectrometry.

1 41. The method of claim 40, wherein the Polysaccharide is selected from the group  
 2 consisting of Polysaccharide 1, Polysaccharide 2, Polysaccharide 3, Polysaccharide 4,  
 3 Polysaccharide 5, Polysaccharide 6, Polysaccharide 7, Polysaccharide 8, Polysaccharide 9,  
 4 Polysaccharide 10, Hexasaccharide 11, Hexasaccharide 12, Hexasaccharide 13,  
 5 Pentasaccharide 14, Pentasaccharide 15, Pentasaccharide 16, and Tetrasaccharide 17.

1 42. A method of 2-*O*-sulfating Polysaccharide 10, comprising the steps of treating  
 2 Polysaccharide 10 with epimerase and 2-OST1.

1 43. The method of claim 1 or 3, comprising the steps of  
 2 (a) treating a heparan synthon with an *N*-sulfating reagent;  
 3 (b) treating the step (a) product with a heparitinase reagent;  
 4 (c) treating the step (b) product with an epimerizing reagent and a 2-*O*-sulfating reagent;  
 5 (d) treating the step (c) product with a 6-*O*-sulfating reagent;  
 6 (e) treating the step (d) product with a  $\Delta^{4,5}$  glycuronidase; and  
 7 (f) treating the step (e) product with 3-*O*-sulfating reagent.

1 44. The method of claim 1 or 3, comprising the steps of  
 2 (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent;  
 3 (b) treating the step (a) product with an epimerizing reagent;  
 4 (c) treating the step (b) product with a 6-*O*-sulfating reagent; and  
 5 (d) treating the step (c) product with nitrous acid and sodium borohydride.

1 45. The method of claim 43 or 44, wherein the products of step (d) are disaccharides.

1 46. The method of claim 1 or 3, comprising the steps of

- 2 (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent; and  
3 (b) treating the step (a) product with an epimerizing reagent and an 2-*O*-sulfating reagent.
- 1 47. The method of claim 1 or 3, comprising the steps of  
2 (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent;  
3 (b) treating the step (a) product with an epimerizing reagent; and  
4 (c) treating the step (b) product with a 2-*O*-sulfating reagent.
- 1 48. The method of claim 1 or 3, comprising the steps of  
2 (a) treating an unsulfated polysaccharide with an *N*-sulfating reagent; and  
3 (b) treating the step (a) product with an 2-*O*-sulfating reagent.
- 1 49. The method of determining the structural identification of Hexasaccharide 11,  
2 comprising the steps of  
3 (a) treating Hexasaccharide 11 with  $\Delta^{4,5}$ - $\beta$ -glucuronidase; and  
4 (b) treating the step (a) product with an  $\alpha$ -*N*-Acetylglucosaminidase.
- 1 50. A method of producing Polysaccharide 2, the method comprising the step of treating  
2 Polysaccharide 1 with an *N*-sulfating reagent.
- 1 51. A method of producing Polysaccharide 3, the method comprising the step of treating  
2 Polysaccharide 2 with an epimerizing agent.
- 1 52. A method of producing Polysaccharide 4, the method comprising the step of treating  
2 Polysaccharide 3 with a 2-*O*-sulfating reagent.
- 1 53. A method of producing Polysaccharide 5, the method comprising the step of treating  
2 Polysaccharide 4 with a 6-*O*-sulfating reagent.
- 1 54. A method of producing Polysaccharide 6, the method comprising the step of treating  
2 Polysaccharide 5 with a 3-*O*-sulfating reagent.
- 1 55. A method of producing Polysaccharide 7, the method comprising the step of treating  
2 Polysaccharide 3 with a 6-*O*-sulfating reagent.
- 1 56. A method of producing Polysaccharide 8, the method comprising the step of treating  
2 Polysaccharide 7 with a 3-*O*-sulfating reagent.

- 1 57. A method of producing Polysaccharide 9, the method comprising the step of treating  
2 Polysaccharide 5 with a 3-*O*-sulfating reagent.
- 1 58. A method of producing Polysaccharide 10, the method comprising the step of treating  
2 Polysaccharide 1 with an *N*-sulfating reagent.
- 1 59. A method of producing Hexasaccharide 11, the method comprising the step of  
2 treating Polysaccharide 10 with a heparitinase reagent.
- 1 60. A method of producing Hexasaccharide 12, the method comprising the step of  
2 treating Hexasaccharide 11 with an epimerase reagent and a 2-*O*-sulfating reagent.
- 3 61. A method of producing Hexasaccharide 13, the method comprising the step of  
4 treating Hexasaccharide 12 with a 6-*O*-sulfating reagent.
- 1 62. A method of producing Pentasaccharide 14, the method comprising the step of  
2 treating Hexasaccharide 13 with  $\Delta^{4,5}$ - $\beta$ -glucuronidase reagent.
- 1 63. A method of producing Pentasaccharide 15, the method comprising the step of  
2 treating Pentasaccharide 14 with a 3-*O*-sulfating reagent.
- 1 64. A method of producing Pentasaccharide 16, the method comprising the step of  
2 treating Pentasaccharide 11 with a  $\Delta^{4,5}$ - $\beta$ -glucuronidase reagent.
- 1 65. A method of producing Tetrasaccharide 17, the method comprising the step of  
2 treating Pentasaccharide 16 with a  $\alpha$ -*N*-Acetylglucosaminidase.